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简 报

Effect of Zinc Deficiency on Blood Cortisol and ACTH Concentrations, Cerebrum Cortex NO Synthase Activity in Rat

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Abstract: The effects of zinc deficiency on the serum cortisol and adrenocorticotrophic hormone (ACTH) concentration, and the cerebrum nitric oxide synthase (NOS) activity in rats were studied. Growing rats were allotted to three groups, which were zinc deficiency (ZD), paired-fed (PF) and zinc supplementation after feeding zinc deficient food for 21 days (ZS). The duration of feed test was 35 days. Compared with PF rats, serum cortisol concentration in ZD ones was significantly increased, whereas serum ACTH concentration and cerebrum NOS activity were significantly decreased. The results suggested that zinc might influence the metabolism of hypothalamic-hypophysial-adrenocortical axis and NOS.

Key words: *Zinc deficiency; Cortisol; ACTH; NO synthase*

Hypothalamic-hypophysial-adrenocortical axis is supposed to play an important physiological role in stress response. Accumulating data also suggested that the axis correlated with learning and memory process despite of different results. Weldhuis & de Wied (1984) indicated that low doses of corticotropin-releasing factor facilitated passive avoidance behavior, whereas high doses had the opposite effect following peripheral administration. de Wied *et al*. (1993) suggested that adrenocorticotrophic hormone (ACTH) facilitate the deficient acquisition of shuttle box avoidance behavior in hypophysectomized rats, delay extinction of shuttle box avoidance behavior and pole-jumping avoidance behavior, and facilitate passive avoidance behavior in intact rats. Conrad *et al*. (1997) indicated that the adrenalectomized (ADX) rats were impaired on spatial recognition memory, whereas treatment with aldosterone, a selective type I receptor agonist, restored spatial recognition memory performance of ADX rats.

Nitric oxide (NO) is a new neurotransmitter with many functions recognized in 1980's. NO and NO synthase (NOS) play a very important physiological role in modulation of learning and memory function. The experiment by Bohme *et al*. (1991) showed that NO might be involved in long-term potentiation, which correlates highly with learning and memory function. Hunot *et al*. (1996) reported that since NO could be involved in the production of free radicals and oxidative stress, it might correlate the neuronal loss in Parkinson's disease.

Hypothalamic-hypophysial-adrenocortical axis and NO both correlate with stress response and learning and memory function. Zinc deficiency is a stress to body too. Our previous experiment (Liu *et al*., 1999) indicated that zinc deficiency significantly decreased learning and memory function. The purpose of the present experiment was to study the effect of zinc deficiency on blood cortisol and ACTH concentration and brain NOS activity so as to explain the neurobiochemical

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mechanism of zinc influencing learning and memory.

1 Materials and Methods

1.1 Animals and diets

Wistar rats (♂ : ♀ = 1 : 1) of about 85 g were randomly allotted to 3 experimental groups which were zinc deficiency group (ZD), fed the low zinc diet; paired-fed group (PF), fed the adequate zinc diet, but restricted to feed consumption weight to the ZD rats; and zinc deficient-supplemented group (ZS), fed adequate zinc diet after feeding the low zinc diet for 21 days. They were housed individually in suspended stainless steel wire-grid cages in a room maintained at (25 ± 2)℃ and 55% humidity with a 12 h light : 12 h dark circle. Deionized water was provided continuously from individual bottles, and food was daily in dry form. The duration of feed test was 35 days. The composition of 10 kg low zinc diet was: 1 900 g soybean protein (treated with 0.5% EDTA to deplete its zinc content); 6 500 g sucrose; 1 000 g soybean oil; 584 g mineral premix (containing: 276 g $CaHPO_4$, 98 g $CaCO_3$, 69 g $NaHPO_4$, 38 g NaCl, 69 g KCl, 29 g $MgSO_4$, 0.1 g KIO_3, 0.1 g $CuSO_4.5H_2O$, 2.0 g $MnSO_4$, 2.5 g $FeSO_4.7H_2O$); 16 g Vitamin premix (containing: 24 mg thiamin.HCl, 60 mg riboflavin, 30 mg pyridoxine.HCl, 200 mg Ca pantothenate, 400 mg niacin, 1 000 mg cycloxanhexol, 20 mg biotin, 20 mg folacin, 0.2 mg cyanocobalamin, 2×10^5 IU retinyl acetate, 4×10^4 IU cholecalciferol, 600 mg menadion, 10 000 mg choline chloride). 1 kg low zinc diet contained 3.3 mg zinc, and 1 kg adequate zinc diet held 100 mg zinc by supplementing $ZnSO_4$.

1.2 Tissue collection

Tissues were collected from six rats of every experimental group on the same day. The animals were decapitated by guillotine, trunk blood was collected and serum was stored in liquid nitrogen for cortisol and ACTH analysis, and cerebrum cortex was collected and stored in liquid nitrogen until used for NOS activity analysis.

1.3 Assay of serum cortisol and ACTH

Radioimmunoassay (RIA) method was used. Cortisol and ACTH assay kits were purchased from Tianjin DPC Biotechologic and Medical Products LMT Company. Assay procedure was based on the direction of the kits.

1.4 Assay of cerebrum cortex NOS activity

Cortex samples were homogenized on ice in 40 mmol/L phosphate-buffered saline (PBS, pH = 7.4), using glass homogenizer. Determining pharmacological agent kit, which mainly contains arginine, NADPH and hemoglibin, was purchased from Institute of Radiation Medicine, Academy of Military Medical Sciences, China. Assay procedure was based on the direction of the kit. NOS activity was calculated as the net synthesis of NO, and expressed as nanomoles of NO per gram tissue per minute.

2 Results

2.1 Concentration of serum cortisol and ACTH

The results of cortisol and ACTH determined were shown in Table 1. The concentration of serum cortisol in ZD group was significantly ($P < 0.01$) higher than in PF, and insignificantly higher than in ZS. The concentration of serum ACTH in ZD rats was significantly ($P < 0.05$) lower than in PF and ZS. Zinc supplementation could reverse the change of serum ACTH and cortisol, however the change of ACTH was quicker than that of cortisol.

Table 1 Effect of zinc deficiency on serum cortisol and ACTH concentrations in rats (M ± SD, $n = 6$)

Group	Cortisol (ng/mL)	ACTH (pg/mL)
ZD	47.5 ± 5.3**	30.0 ± 2.7*△
PF	21.1 ± 4.5	46.5 ± 3.7
ZS	40.6 ± 4.0*	46.0 ± 4.0

* $P < 0.05$, ** $P < 0.01$, compared with PF; △ $P < 0.05$, compared with ZS

ZD refers to consumption of the low zinc diet, PF to restricted consumption of the adequate zinc diet, ZS to restricted consumption of the adequate zinc diet after consuming the low zinc diet for 21 days.

2.2 NOS activity in cerebrum cortex

The result of NOS activity determined was shown in Fig. 1. NOS activity of cerebrum cortex in ZD group [(0.57 ± 0.15) nmol·(min·g)$^{-1}$] was significantly ($P < 0.05$) lower than that in PF [(1.01 ± 0.09) nmol·(min·g)$^{-1}$].

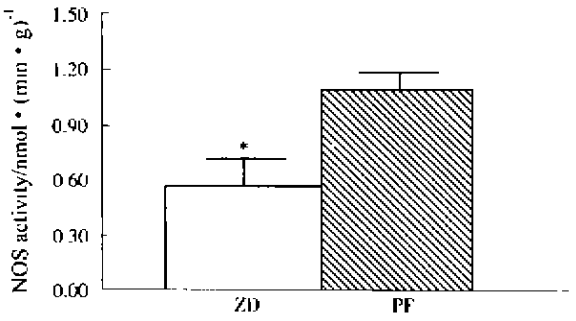

Fig. 1 Effect of zinc deficiency on nitric oxide synthase (NOS) activity of cerebrum cortex in rat

* $P < 0.05$, ZD compared with PF, $n = 6$.

3 Discussion

The increase of glucocorticoid secretion is one index of stress response in body. The significant increase of serum cortisol in zinc deficient rats in the present experiment suggested that long-term zinc deficiency could result in serious stress of rats. Conrad *et al*. (1997) indicated that spatial memory and exploratory behavior ability of adrenalectomized rats was significantly decreased, while applying corticosteroid receptor agonists might modulate their memory and exploratory behavior ability to normal status. Kirschbaum *et al*. (1996) reported that stress and drug-induced elevation of cortisol levels impaired explicit memory in healthy adults. It was indicated that zinc deficiency significantly decreased learning and memory ability of rats in our previous experiment (Liu *et al*., 1999), whereas increased their serum cortisol levels in the present experiment. These results suggested that glucocorticoid played an important role in modulating learning and memory function.

ACTH, which is a hormone secreted by pituitary gland, can stimulate secretion of adrenocortical hormone, and meanwhile the latter has a negative feedback effect on secretion of the former. The result in the present experiment, which zinc deficiency resulted in significant increase of blood cortisol concentration, whereas decrease of blood ACTH concentration, also suggested the negative feedback effect of cortisol on ACTH secretion in pituitary. Some data indicated that abnormal behavior was shown in pituitectomized rats. Another data indicated that ACTH analog, which couldn't influence the secretion of glucocorticoid in adrencortex, could also increase the maintenance of memory. Zhang *et al*. (1996) reported that learning and memory ability were significantly increased because of intrahippocampally injected ACTH. These data suggest that hippocampus could be an important site for ACTH-facilitated learning, and ACTH could modulate learning and memory. The results of the present experiment and our previous experiment (Liu *et al*., 1999) suggested decrease of blood ACTH concentration in zinc deficient rats might have some active effect to prevent decrease of learning and memory ability caused by zinc deficiency.

NOS plays a role in leading to conversion from L-arginine to L-citrulline and the production of NO. NO is supposed to have extensive role, which has been identified as a very important local and retrograde messenger in central nervous system. The role of NO and NOS in various forms of learning and memory has been established. Bohme *et al*. (1991) indicated that NO involved in hippocampal long-term potentiation (LTP), which correlated highly with learning and memory function. Liu *et al*. (1996) pointed that intraseptally injecting low dose of sodium nitroprusside, which can automatically produce NO in body, increased learning and memory ability, whereas injecting the inhibitor of NOS decreased learning and memory ability of rats. Myslivecerk *et al*. (1996) reported that NO exerted a considerable central modulatory effect on learning, memory processing and retrieval at the very early postnatal period of the rat. The present experiment showed that zinc deficiency decreased cerebrum cortex NOS activity in rats, and this was similar to the result of Xie *et al*. (1995), in which zinc deficiency decreased the NOS activity of cerebellum, striatum and hypothalamus in mice. These results suggested that low learning and memory ability caused by zinc deficiency might correlate with the decrease of NOS activity and NO production.

In conclusion, the effect of zinc deficiency on learning and memory ability of rats correlated with the hypothalamic-hypophysial-adrenocortical axis, and al-

so with NO. However, the relation between the hypothalamic-hypophysial-adrenocortical axis and NO is still needed to study.

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缺锌对大鼠血液皮质醇和 ACTH 含量及大脑皮质 NO 合酶活性的影响

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摘要：就缺锌对大鼠血液皮质醇和促肾上腺皮质激素（ACTH）含量以及大脑皮质 NO 合酶活性的影响进行了研究。生长大鼠随机分为 3 组，即缺锌组、对喂组和缺锌补锌组（先饲喂缺锌饲料 21 天后再补锌）。饲养实验的持续时间为 35 d。与对喂组比较，缺锌组大鼠血液中皮质醇含量显著升高，而血液 ACTH 浓度以及大脑皮质 NO 合酶活性明显降低。此结果提示锌可影响下丘脑—垂体—肾上腺皮质轴和 NO 合酶的代谢。

关键词：缺锌；皮质醇；ACTH；NO 合酶

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